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A controlled trial of sputum induction and routine collection methods for TB diagnosis in a South African community

Hennie D. Geldenhuys^{1,2}, Andrew Whitelaw³, Michele D. Tameris^{1,2}, Danelle Van As^{1,2}, Kany Kany A. Luabeya^{1,2}, Hassan Mahomed^{1,2}, Gregory Hussey^{1,2}, Willem A. Hanekom^{1,2}, and Mark Hatherill^{1,2}

¹South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa

²School of Child and Adolescent Health, University of Cape Town, South Africa

³Division of Medical Microbiology, Faculty of Health Sciences, University of Stellenbosch, South Africa National Health Laboratory Service, Tygerberg Hospital, Cape Town, South Africa

Abstract

BACKGROUND—Diagnostic yield of pulmonary tuberculosis (TB) by sputum induction (SI) at first point of contact with health services, conducted in all patients with suspected TB regardless of ability to expectorate spontaneously, has not been evaluated. We compared diagnostic yield of SI to routine sputum collection in a South-African community setting.

METHODS—Ambulatory patients with suspected TB provided a ‘spot’ expectorated sputum sample; an SI sample by hypertonic (5%) saline nebulization; and early morning expectorated sputum sample. Diagnostic yield of sputum smear microscopy and liquid culture (denominator all subjects with any positive *Mycobacterium tuberculosis* culture), and time-to-positivity of culture were compared between SI and expectorated samples.

RESULTS—555 subjects completed the SI procedure, of whom 132 (24%) were HIV-infected. One-hundred-twenty-nine samples (23%) were *M. tuberculosis* culture positive. Time-to-positivity of MGIT culture was shorter for SI (median difference 2 days, $p=0.63$), and for early morning expectorated sputum (median difference 2 days, $p=0.02$), compared to spot expectorated sputum. However, there was no difference in culture-positive diagnostic yield between SI and spot expectorated sputum (difference +0.7%; CI −7.0 to +8.5%, $p=0.82$), or SI and early morning expectorated sputum (difference +4.7%; CI −3.2 to +12.5%, $p=0.20$) for all subjects; or for HIV-infected subjects.

Corresponding author: Dr. Hennie Geldenhuys, SATVI, Brewelskloof Hospital, Haarlem Street, Worcester, 6850. hennie.geldenhuys@uct.ac.za. telephone: +27 23 3465400. **Alternate corresponding author:** Prof. Mark Hatherill, SATVI, IDM, Werner Beit Building, Anzio Road, UCT, Cape Town. mark.hatherill@uct.ac.za. telephone: +27 21 404 7618.

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All authors declare that they have no conflict of interest.

The study was approved by the University of Cape Town Human Research Ethics Committee (Human Subjects Assurance number 00001637) and written informed consent was obtained for participation. The study was conducted in accordance with the Declaration of Helsinki.

CONCLUSIONS—SI reduces time to positive *M. tuberculosis* culture, but does not increase the rate of positive culture, compared to routine expectorated sputum collection. SI cannot be recommended as the routine collection method at first contact among ambulatory patients with suspected TB in high burden communities.

Keywords

induced sputum; tuberculosis; diagnostic yield; mycobacterium

INTRODUCTION

Rapid, reliable diagnosis of tuberculosis (TB) disease is key to control of the epidemic. Microbiological confirmation of *Mycobacterium tuberculosis* (MTB) by liquid culture is the gold standard of TB diagnosis and collection of at least two expectorated sputum specimens is established practice [1, 2]. New and improved diagnostic methods for rapid molecular detection of pulmonary TB are also dependent on obtaining an optimum sputum sample from the respiratory tract [3, 4].

Sputum induction (SI) by inhalation of hypertonic saline is an alternative to routine expectorated sputum collection methods [5–7]. SI may increase the yield of microbiological diagnosis in patients with suspected TB, by stimulating increased volume of sputum expectoration in otherwise nonproductive patients, or by improving the quality of the sample by increasing the number of viable mycobacteria in sputum productive patients with suspected TB [8]. Diagnosis by SI may be especially relevant in primary healthcare settings in developing countries, where sophisticated diagnostic modalities, such as bronchial lavage, are unavailable, and the burden of TB is high [5].

Hospital-based studies have shown that paediatric and adult patients with suspected TB who do not produce sputum spontaneously, or who are sputum negative despite clinical suspicion at the first point of contact with health services, benefit from SI [5, 9–14]. The majority of these studies have evaluated SI as a confirmatory diagnostic modality after sputum collection by routine expectoration – the current standard of care – has failed to produce a positive result. It is unclear whether SI might improve diagnostic yield when used as a sputum collection method at the first point of contact, instead of routine expectorated collection, among all patients with suspected TB in a community with a high burden of TB.

An improvement in diagnosis of TB by SI at the first point of contact in a high-burden community setting has the potential to diagnose more cases earlier in TB disease progression, and thus decrease transmission, but this hypothesis can only be evaluated by head-to-head comparison of diagnostic yield with routine expectorated sputum collection methods. We have shown previously in a community setting that SI is safe and well tolerated in ambulatory patients with suspected TB [15]. We aimed to quantify the potential benefit offered by first-line SI in this study population, compared to spontaneous expectorated sputum collection methods, using a direct, within-subject comparison of microbiological diagnostic yield.

METHODS

Participants and Setting

We compared the microbiological diagnostic yield of *M. tuberculosis* between sputum samples obtained by SI and sputum samples obtained by routine expectorated sputum collection methods, in ambulatory patients aged 12 years and older who presented to community clinics with suspected pulmonary TB. The study was performed in a rural community near Worcester, South Africa, where the incidence of TB disease is high (1030/100,000) [16]. The study was approved by the University of Cape Town Human Research Ethics Committee (Human Subjects Assurance number 00001637) and written informed consent was obtained for participation. The study was registered on [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00608790) (NCT00608790).

Patients with suspected TB, based on symptoms suggestive of pulmonary TB, including cough of at least 2 weeks duration, weight loss, night sweats, or loss of appetite, were recruited after presentation to the clinic. Exclusion criteria included current or prior treatment for TB, or a history of asthma, heart disease, cardiac arrhythmia, pregnancy or lactation, treatment with tricyclic anti-depressants, acute or chronic lung disease that could potentially compromise lung function, or a need for supplemental oxygen therapy at the time of screening. TB treatment should not have been initiated more than 72 hours before enrolment. All participants were tested for HIV infection and both HIV negative and HIV positive participants were recruited irrespective of ARV treatment. CD4 counts were not determined as part of the study protocol.

Sputum collection procedures

Three sputum samples were collected from each participant: a 'spot' expectorated sputum sample; an induced sputum (SI) sample at least 4 hours later on the same day; and an early morning expectorated sputum sample on the following day. The proper technique for expectorated sputum collection was demonstrated to the participant by the study team. Participants were instructed to take a deep breath, hold their breath for a moment, cough deeply and vigorously when breathing out, and to expectorate into the labelled container by holding the jar to the lower lip and gently releasing the sputum specimen. It was explained that thick mucoid sputum, and not saliva or nasal secretions, should be collected. Four hours later, participants underwent an SI procedure with hypertonic 5% saline nebulization preceded by beta-2 agonist (100–200 mcg albuterol) inhalation. The sputum induction procedure was performed by a trained registered nurse in an out-patient procedure room of the regional TB hospital. Resuscitation and monitoring equipment were available and standard infection control measures, including a high flow extractor fan and N95 particulate filter masks, were used [15]. Participants fasted for at least 3 hours before the start of the SI procedure. Immediately prior to the procedure, a baseline clinical assessment was done that included a physical examination and recording of vital signs. Participants were monitored for adverse events throughout the procedure and for at least 30 minutes after the procedure. Thirty (30) mL 5% hypertonic sodium chloride solution was administered by nebulization via face-mask with 100% oxygen at a flow-rate of 15 litres per minute for up to 20 minutes, or until all of the saline had been nebulized. Sputum was collected at 5 minute intervals

during the nebulization and at any time during the procedure when the participant felt an urge to cough. Participants were then provided with a specimen collection jar and instructed to follow the expectorated sputum collection procedure for the early morning sputum sample on the following day.

Mycobacterial culture

Sputum samples were transported on the same day as collection to the research microbiology section of the National Health Laboratory Services (NHLS) in Cape Town and underwent initial processing on the same day. Samples were transported at room temperature in secure biohazardous containers. Samples were liquefied and decontaminated using N-acetyl-L-cysteine and NaOH (final concentration 1.5%). A portion of the decontaminated pellet underwent smear microscopy using Auramine staining, and the remainder was inoculated into Mycobacterial Growth Indicator Tube (MGIT) (Becton Dickinson, USA), and incubated for up to 42 days. Positive mycobacterial cultures were confirmed as being *Mycobacterium tuberculosis* complex, as well as tested for susceptibility to isoniazid (INH) and rifampicin (RIF) using the Hain MTBDRplus line probe assay according to the manufacturer's instructions. (Hain Lifescience, Germany). These results were confirmed by phenotypic susceptibility testing (for RIF and INH) in the MGIT system. The number of viable bacteria present in samples with positive MGIT cultures was evaluated by recording time to positive culture (days) as a proxy measure. An initial report of a contaminated culture was not revised by an attempt at re-decontamination. Results of microscopy and culture were provided to the participant and to the nurse at the referring community clinic. Where indicated, treatment was provided by the clinic per national treatment program guidelines.

Data analysis

Data were entered into MS-Access (2003) and Stata (version 11 StataCorp 2009). Participants who were enrolled and completed the induced sputum (SI) procedure, and whose sputum results were available, and whose MGIT cultures were not contaminated, and who had at least one positive mycobacterial culture, were included in this analysis.

The diagnostic yield by sputum smear microscopy and by MGIT culture, and the proportion of collection attempts that produced a sputum sample, were compared between induced sputum, spot expectorated sputum, and early morning expectorated sputum collection methods. The denominator for diagnostic yield calculation was the number of patients with a positive *M. tuberculosis* culture by any collection method (ie. all microbiologically confirmed TB cases). The proportion of positive MGIT cultures by each individual collection method, and the difference in diagnostic yield between sputum collection methods were calculated. The 95% confidence interval (CI) and statistical significance of the difference in proportions were determined using McNemar's test for paired samples. Comparisons between collection methods for time to MGIT culture positivity were made using median difference (days) and the Kruskal Wallis test for non-parametric data. A p value 0.05 was considered statistically significant.

RESULTS

Six hundred patients with suspected TB were enrolled. Median age was 30 years (IQR 23–41), 301 participants (50%) were female and 132 (24%) were HIV infected. Forty-one participants (7%) were assessed as unfit for the SI procedure at baseline: 17 (41.5%) due to abnormal vital signs, 12 (29.3%) for low baseline oxygen saturation, 8 (19.5%) for shortness of breath, 3 (7.1%) were not fasting, and 1 (2.4%) withdrew consent prior to procedure start. Of the remaining 559 who started the procedure 4 (0.7%) did not complete the procedure for safety reasons. Of the 555 participants who completed the procedure, 554 (99.8%) were productive of sputum by at least one collection method and 465 (83.8%) productive by all 3 collection methods. On spot sputum alone 536 (96.6%) were sputum productive, 545 (98.2%) on induced sputum, and 532 (95.9%) on early morning sample. Four results were not available for analysis, 52 samples were not collected because the participant was non-productive of sputum, and 80 were contaminated, leaving 1529 individual samples available for analysis (Table 1).

There was no difference in the proportion of successful, productive sputum collection attempts between SI and either of the expectorated sputum collection methods (98% vs 96%, $p>0.1$), irrespective of HIV status. One hundred and twenty nine participants (129) had a positive MTB culture from at least one collection method (23.2 % of 555 who completed the SI procedure) and 302 (19.8%) individual culture specimens were positive, constituting the denominator for all calculations of diagnostic yield (Table 1). No cases of MTB resistance were detected.

Sputum smear microscopy

The difference in diagnostic yield of sputum smear microscopy between SI and early morning sputum collection did not reach statistical significance (difference +3.9% $p=0.25$). However, diagnostic yield of sputum smear microscopy was significantly greater for SI only among HIV positive participants (SI vs early morning smear difference 17.6 % CI 0.1 to +35.8 %; $p=0.05$). The diagnostic yield of sputum smear microscopy did not differ between SI and spot expectorated sputum specimens (difference +0.7% $p=0.82$) (Tables 2 and 3).

MGIT culture

Compared to a single spot expectorated sputum collection, SI confirmed an additional 15 MTB culture positive TB cases; and early morning expectorated sputum confirmed an additional 16 cases. However, the diagnostic yield of MGIT culture was not significantly greater for SI compared to spot expectorated sputum (difference +2.3%; $p=0.51$), or for SI compared to early morning expectorated sputum (difference = +3.1% $p=0.47$), among all participants. Similarly, the diagnostic yield of MGIT culture was not significantly greater for SI compared to spot expectorated sputum (difference +11.8%; $p=0.1$), or for SI compared to early morning expectorated sputum (difference +5.9%; $p=0.48$), among HIV positive participants only.

Time to positive MGIT culture

Positive MGIT culture sputum samples obtained from HIV negative participants by any method had a significantly shorter Time to Positivity (TTP) than HIV positive participants (difference -3 days, $p=0.021$), consistent with a greater load of viable MTB organisms (Figure 1). Compared to spot expectorated sputum, time-to-positivity on MGIT culture was shorter from early morning expectorated sputum (median difference 2 days, $p=0.02$) and from SI (median difference 2 days, $p=0.63$).

Safety and tolerance

There were no serious adverse events (SAEs). Eleven non-serious adverse events occurred (Table 4) in which the SI procedure was terminated in four cases. All adverse events were mild, self-limiting, and required no treatment.

DISCUSSION

We found no increase in diagnostic yield of MGIT culture using SI by 5% hypertonic saline by face mask nebulization, compared to routine expectorated sputum collection methods, at the first point of contact with health services, in ambulatory patients with suspected TB in a high burden South African community. We observed increased diagnostic yield of sputum smear microscopy by SI, compared to early morning expectorated sputum, but among HIV infected participants only. The number of viable MTB organisms in the sputum sample, as shown by shorter time to positive MGIT culture, was higher in samples collected by SI, and also by early morning expectorated sputum collection, compared to spot expectorated sputum. The SI procedure was safe and well tolerated, in keeping with our earlier results in this study population [15]. Although there was no increase in diagnostic yield by sputum induction cumulative yield was increased by taking more than one sample for culture by any collection method.

These findings differ from previous studies that suggest SI increases diagnostic yield in adult patients with suspected TB [4, 5, 9–12]. The difference is likely due to the fact that this study population was not pre-screened by inability to produce a sputum sample, or by a negative result from routine expectorated sputum samples. We included all patients with suspected pulmonary TB, based on first presentation with suggestive symptoms, prior to radiological or microbiological investigations. The overwhelming majority of our participants were able to produce sputum spontaneously (96.6% on spot sample alone), whereas previous studies were conducted amongst patients with a high risk of TB, but who could not produce sputum spontaneously, or who were sputum smear or culture negative on routine expectorated sputum samples, and in whom SI was performed as a second-line confirmatory collection procedure.

Our findings suggest that sputum induction by hypertonic saline adds little value to TB diagnosis at the first point of contact with health services in a high burden community where most TB suspects are spontaneously productive of sputum. The modest increase in viable MTB load by SI did not translate into greater diagnostic yield, presumably because this study population, i.e. all patients with suspected TB, included few individuals with

paucibacillary disease. It is possible that increased diagnostic yield might be observed if SI were used as the primary sputum collection procedure in selected study populations with paucibacillary disease, such as children or HIV infected patients [13]. The potential value of SI as the primary collection method for improved TB diagnosis in HIV infected patients, implied by the increased diagnostic yield by smear microscopy, would require a larger sample size to evaluate. Similarly, SI at the first point of contact with health services at community level might offer diagnostic benefit in asymptomatic, or early symptomatic patients with paucibacillary TB disease.

Contrary to our expectation, diagnostic yield from sputum smear microscopy and liquid culture was not greater in early morning expectorated sputum samples compared to spot samples. This is surprising, if we consider the accepted paradigm that expectorated sputum specimens taken in the early morning produce better yield than spot sputum collection [1].

A greater proportion of samples were contaminated amongst the induced sputum and early morning samples compared to spot samples (1.6%, 5.7%, 7.5% respectively; $p=0.005$). However, the overall rate of contamination was low (80/1609 samples; 5%) and is unlikely to influence the findings of our analysis. There is not an obvious explanation for this difference, other than the limitation of our study design that required the early morning specimens to be collected at participant's homes and stored there in potentially less optimal conditions until they were transported to the laboratory. This is likely to be a common scenario in resource limited settings.

The load of viable MTB organisms in sputum, as measured by time to positive MTB culture in liquid medium, has been proposed as a predictor of TB treatment success and as a potential endpoint in clinical trials for early biomarkers of treatment response and relapse [17, 18]. We have shown that the sputum collection method influences time to MGIT culture positivity, and we infer, load of viable MTB organisms, which is greatest in early morning and lowest in spot expectorated sputum samples. It follows that sputum collection method should be considered when comparing bacillary load findings across clinical trials.

We acknowledge that, since participants who were judged unfit to undergo SI were excluded from participation, these findings may not be generalizable to TB patients with a more severe spectrum of disease, for example, patients in hospital settings. However, we suggest that SI would be expected to show least relative benefit among patients with severe PTB disease and greater number of viable MTB organisms in the sputum. Other limitations of the study include the fact that we did not collect data on the volume of sputum collected, nor the subjective quality of the specimen, which might correlate with MTB culture yield. The SI procedure is not a standardised technique and it is possible that variations in saline tonicity, saline volume, or nebulization device might produce different results [7].

CONCLUSION

SI was safe and feasible, but did not increase diagnostic yield of MGIT culture compared to routine sputum collection methods in ambulatory patients with suspected TB, the majority of whom were able to produce a spontaneous expectorated sputum sample. The number of

viable MTB organisms in the sample, measured by time to positivity of MGIT culture, was greater in SI samples, and also in early morning expectorated samples, compared to spot sputum samples. However, increased number of viable organisms in the sample by SI did not translate into improved diagnostic yield among all patients, presumably due to the inclusion of few participants with paucibacillary disease. In HIV-infected participants only, diagnostic yield of sputum smear microscopy was marginally greater by SI than by early morning expectorated sputum collection. Based on these findings, and considering cost and logistical factors, SI cannot be recommended as the primary sputum collection method at the first point of contact for ambulatory patients with suspected TB in high burden countries. The potential benefit of SI as a first-line sputum collection method for selected patient populations with pauci-bacillary disease, such as HIV co-infected persons, remains to be evaluated.

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¹South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine, and ²School of Child and Adolescent Health, University of Cape Town, South Africa

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Figure 1.

Time to positive MTB culture (TTP) from samples obtained by spot expectorated sputum, induced sputum, and early morning expectorated sputum. Time to culture is shown in median days (horizontal line), interquartile range (box), and values within $1.5 \times \text{IQR}$ (whiskers). Text labels show medians (interquartile range).

(a): Median TTP by sample collection method (by HIV uninfected and HIV infected participants)

(b): Median TTP by sample collection method (all participants irrespective of HIV status). TTP was significantly longer for HIV infected compared to HIV uninfected participants (median difference 3 days, $p=0.021$, all collection methods); and significantly longer for spot expectorated sputum samples compared to early morning samples (median difference 2 days, $p=0.020$, HIV + and HIV -). TTP from spot samples was also longer than SI samples (median difference 2 days, $p=0.63$). TTP did not differ significantly between SI and early morning samples (median difference=0 days, $p=0.10$)

Table 1

Summary of number of sputum specimens obtained by collection method and HIV status. Total samples analysed are all samples for which a result was available (positive, negative, contaminated). For samples with results available n% is shown as proportion of total samples analysed. For lost specimens/results and cases where no sputum could be obtained, n% is shown as a proportion of all cases where all 3 collection methods were performed.

	All (N=555)	HIV+ (N=118)	HIV- (N=437)
SPOT			
Smear Result			
Positive	66 (12.3%)	9 (7.8%)	57 (13.5%)
Negative	470 (87.7%)	106 (92.2%)	364 (86.4%)
Total samples analysed	536	115	421
No sputum produced	19 (3.4%)*	3 (2.5%)*	16 (3.7%)*
Culture Result			
Positive	98 (18.3%)	22 (19.1%)	76 (18.1%)
Negative	427 (80.0%)	89 (77.4%)	338 (81.1%)
Contaminated	9 (1.6%)	4 (3.4%)	5 (1.1%)
Total samples analysed	534	115	419
Lost specimen/result	2 (0.3%)*	0	2 (0.5%)
No sputum produced	19 (3.4%)*	3 (2.5%)*	16 (3.7%)*
INDUCED SPUTUM			
Smear Result			
Positive	77 (14.1%)	15 (12.8%)	62 (14.5%)
Negative	468 (85.9%)	102 (87.2%)	366 (85.5%)
Total samples analysed	545	117	428
No sputum produced	10 (1.8%)*	1 (0.8%)*	9 (2.0%)*
Culture Result			
Positive	105 (19.3%)	24 (20.5%)	81 (19.0%)
Negative	407 (75.0%)	88 (75.2%)	319 (74.9%)
Contaminated	31 (5.7%)	5 (4.3%)	26 (6.1%)
Total samples analysed	543	117	426
Lost specimen/result	2 (0.3%)*	0	2 (0.5%)
No sputum produced	10 (1.8%)*	1 (0.8%)	9 (2.1%)
EARLY MORNING			
Smear Result			
Positive	65 (12.2%)	8 (7.1%)	57 (13.6%)
Negative	467 (87.8%)	105 (92.9%)	362 (86.4%)
Total samples analysed	532	113	419

	All (N=555)	HIV+ (N=118)	HIV- (N=437)
No sputum produced	23 (4.1%)*	5 (4.2%)	18 (4.1%)
Culture Result			
Positive	99 (18.6%)	21 (18.6%)	78 (18.6%)
Negative	393 (73.9%)	79 (69.9%)	314 (74.9%)
Contaminated	40 (7.5%)	13 (11.5%)	27 (6.4%)
Total samples analysed	532	113	419
Lost specimen/result	0	0	0
No sputum produced	23 (4.1%)*	5 (0.9%)	18 (4.1%)

Table 2

Difference in diagnostic yield between collection methods for all participants irrespective of HIV status (N= 129 positive culture by at least 1 method).

	Sputum induction vs Spot	Sputum Induction vs Early Morning	Spot vs Early Morning
Smear			
Difference in yield (95% CI)	0.7% (−7.0 8.5)	4.7% (−3.2 12.5)	3.9% (−3.4 11.2)
p-value	0.82	0.20	0.25
Positive by both methods	65 (50.4%)	62 (48.1%)	63 (48.8%)
Negative by both methods	43 (33.3%)	45 (34.9%)	47 (36.4%)
Culture			
Difference in yield (95% CI)	2.3% (−5.4 10.0)	3.1% (−5.9 12.1)	0.8 % (−7.9 9.4)
p-value	0.51	0.47	0.84
Positive by both methods	100 (77.5%)	95 (73.6%)	95 (73.6%)
Negative by both methods	8 (6.2%)	4 (3.1%)	7 (5.4%)

Table 3

Difference in diagnostic yield between collection methods for HIV participants (N= 34 positive culture by at least 1 method).

	Sputum induction vs Spot	Sputum Induction vs Early Morning	Spot vs Early Morning
Smear			
Difference in yield (95% CI)	11.8 % (−4.7 28.3)	17.6% (0.1 35.8)	5.9 % (−13.2 25.0)
p-value	0.10	0.05	0.48
Positive by both methods	12 (35.3%)	10 (29.4%)	8 (23.5%)
Negative by both methods	16 (47.1%)	16 (47.1%)	18 (52.9%)
Culture			
Difference in yield (95% CI)	0% (−0.2 19.2)	2.9 % (−20.7 26.7)	2.9 % (−19.1 25.0)
p-value	1	0.78	0.76
Positive by both methods	22 (64.7%)	20 (58.8%)	21 (61.8%)
Negative by both methods	4 (11.8%)	1 (2.9%)	2 (5.9%)

Table 4

Summary of adverse events (AE). There were no Serious Adverse Events (SAE). All AEs were self-limiting and resolved spontaneously. AE occurrence is shown by phase of the sputum induction procedure (after albuterol and before saline nebulization, during saline nebulization, or after saline nebulization).

Adverse Event	Number events	Phase of procedure	Duration (minutes)	Comments
Decrease in oxygen saturation < 96%	3	Post-albuterol, pre-nebulization	1	Procedure completed, minimum oxygen saturation = 84%
		Post-nebulization	7	Procedure complete, minimum oxygen saturation = 85%
		Post-albuterol, pre-nebulization	45	Procedure stopped, minimum oxygen saturation = 87%
Rapid or laboured breathing	2	Nebulization [‡]	18	Procedure stopped
		Post-nebulization	15	*N/A
Pain or discomfort	2	Nebulization	3	Procedure stopped
		Post-nebulization	15	N/A
Paroxysmal coughing	1	Nebulization	3	Procedure stopped
Heart rate >135/minute	1	All phases		Elevated at baseline, procedure completed
Vomiting	1	Nebulization [‡]	3	Procedure completed
Hypotension	1	Post-albuterol, pre-nebulization	2	Procedure completed ([†] BP=72/55)

* N/A= not applicable

[†] BP=blood pressure

[‡] 1 participant had 2 AEs, procedure was stopped for rapid/laboured breathing